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The subject of these studies is the development of targeted vector for selective infection and killing prostate cancer cells. The resultant vector is to be administered to prostate cancer patients to find, infect and destroy tumor cells. Targeting of the vector to prostate tumors is to be accomplished via incorporation into its capsid of ligands, which selectively bind to prostate-specific membrane antigen (PSMA).

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INTRODUCTION

The subject of the present studies is the development of phage-based vector capable of selectively infecting and killing prostate cancer cells. The intended use of such a vector is for gene therapy of prostate cancer patients, whereby the phage administered to patients would find, infect and destroy tumor cells. Development of this vector is to employ the concept of genetic targeting of vector to tumor-specific cell surface molecules. Targeting of the vector to prostate tumor cells is be accomplished via genetic incorporation into its capsid of single-chain antibody, which selectively bind to a major marker of prostate tumors, prostate-specific membrane antigen (PSMA). The selectivity of the designed vector for PSMA-positive cells and the efficacy of the cell killing is to be assessed *in vitro*.

REPORT BODY

Objectives

In Year 3 of the project the following research objective were to be achieved:

- To modify the genome of the previously designed PSMA-targeted phage to express a light reporter (green fluorescent protein, GFP) and a therapeutic gene (herpes simplex virus thymidine kinase, HSV tk) in mammalian cells
- To demonstrate the capacity of the phage to transduce PSMA-positive human cells by virtue of binding to PSMA and exploiting its natural capacity for internalization.
- To demonstrate the therapeutic utility of this vector by selectively eradicating PSMA-expressing cells *in vitro*.

Results

The genome of the previously designed phage vector, which was targeted to the extracellular domain of PSMA by incorporating into its virion of a single chain antibody (scFv) C6C, was further modified to contain a gene cassette that expressed either the humanized version of the green fluorescent protein (hrGFP), or the herpes simplex virus thymidine kinase (HSV tk). While the virions of both vectors were identical and both contained the same targeting moiety, C6C scFv, each of the two vectors was designed to serve a different purpose. Specifically, the phage fuCT/C6C/hrGFP was designed for the sole purpose of tumor cell imaging, the fuCT/C6C/TK phage was developed as a therapeutic vector with a self-imaging capability. In the latter instance, the HSV tk expressed by the phage could be employed either as a prodrug-converting enzyme in the context of the gancyclovir(GCV)-mediated therapy, or as a radiolabeled precursor-converting enzyme thereby facilitating the PET-based imaging.

In Year 1 of the project we demonstrated the ability of a phage particle bearing a C6C scFv to selectively bind the PSMA and the PSMA-expressing cells (see our progress report). In addition to those data, we have now confirmed that such a phage vector can

also enter a PSMA-positive cell by using PSMA as a surrogate receptor. Most importantly, the gene payload carried by a targeted phage was expressed within the transduced cells allowing for either the visualization of the cell by optical imaging (due to GFP fluorescence) (Fig. 1), or killing those cells in the presence of GCV (Fig. 2).

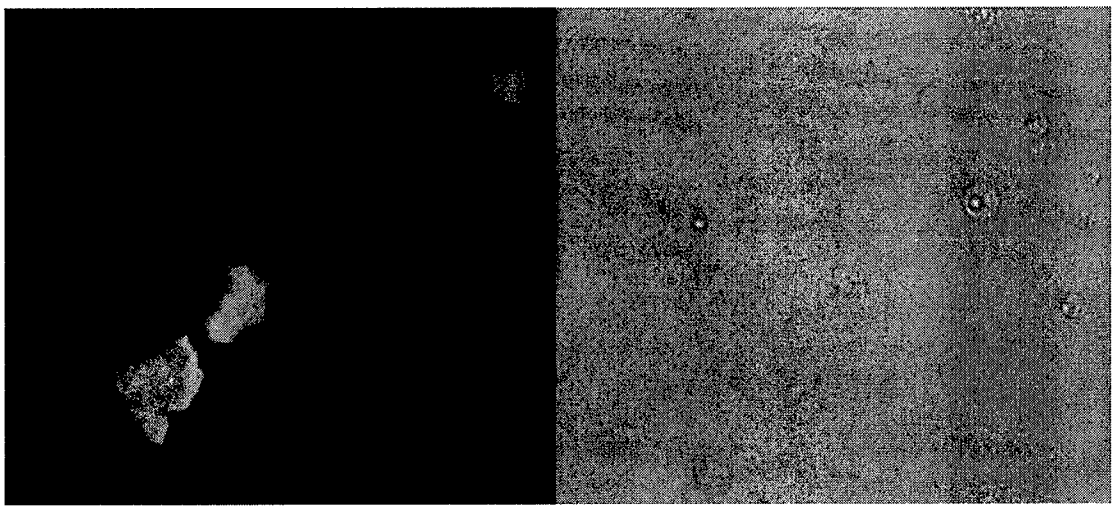

Figure 1. Transduction of PSMA-positive human cells with scFv-targeted phage vector. The derivative of the human embryonal kidney cell line 293 that has been designed to stably express human PSMA, 293/PSMA, was transduced by the hrGFP-expressing, PSMA-targeted phage vector fuCT/C6C/hrGFP. Forty-eight hours post-transduction the cells were examined under the fluorescent microscope. Left panel, fluorescent image; right panel, the same field viewed under white light. No fluorecent foci could be seen in a similarly transduced monolayer of parental 293 cells that are PSMA-negative.

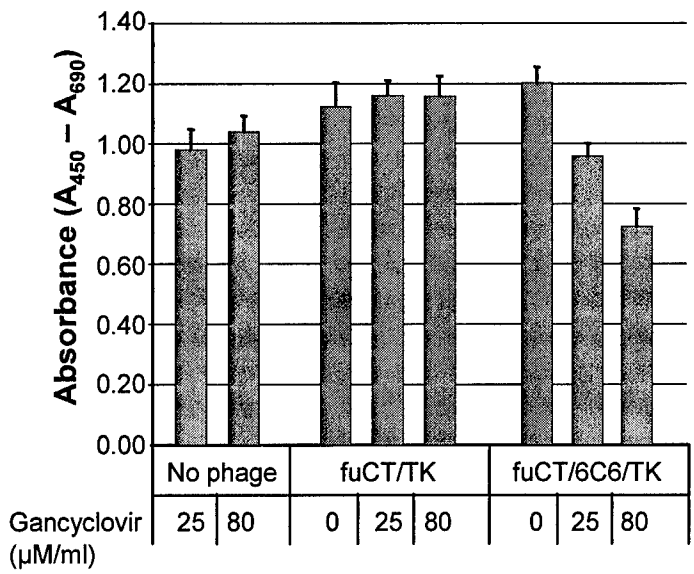

Figure 2. GCV dose-dependent killing of PSMA-positive cells transduced with the HSV tk-expressing, PSMA-targeted phage vector. 293/PSMA cells seeded in a 96-well plate were incubated for 4 h with the fuCT/C6C/TK phage (10^5 phage particles per cell). In thirty-six hours, GCV was added to cells at concentrations of either 25 or 80μM. The cell proliferation rate was assessed using Quick Cell Proliferation Assay Kit (BioVision) five days later.

KEY RESEARCH ACCOMPLISHMENTS

- The genome of the previously designed PSMA-targeted phage has been modified to express a light reporter (green fluorescent protein, GFP) and a therapeutic gene (herpes simplex virus thymidine kinase, HSV tk) in mammalian cells
- The capacity of the targeted phage to transduce PSMA-positive human cells by virtue of binding to PSMA and exploiting its natural capacity for internalization has been shown.
- The ability of the PSMA-targeted, HSV tk-expressing phage to kill PSMA-positive cells has been demonstrated.

REPORTABLE OUTCOMES

In addition to the cell lines, hybridomas, and antibodies that were reported previously, we have now made two new phage vectors that are targeted to PSMA and carry transgene payloads.

CONCLUSIONS

While, in essence, we have demonstrated all the key feasibilities of vector targeting to PSMA and gene delivery, this project would benefit quite significantly from additional experiments that would focus more on the quantitative aspects of the phage-mediated gene transfer. Having such additional data would make the results of our studies more convincing and reliable. This is exactly what we are planning to do in the next few months while the project is in a "no cost extension" phase.

REFERENCES

None

APPENDICES

None